

Article

# Study the Effect of Lemon Extract on Some Types of Bacteria and Candida

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**Abstract:** Extracts of Lemon had long been known for their antimicrobial activities, making Lemon a subject of interest in the scientific research. The current study deals with investigating the impacts of lemon peels extracts (ethanol and chloroform) on *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Candida albicans* (*C. albicans*), assessing their inhibitory effects via MIC, IC<sub>50</sub> values, and optical densities (ODs) measurements. The phytochemical examination of lemon peels extract revealed that these peels have different bioactive compounds at different levels, the concentration of total polyphenol was 124 (mg/L), Flavonoids was at 150 (mg/L), Tannins level was 13 (mg/L), while alkaloids were found with low concentration at 5 (mg/L). The current findings indicate a strong inverse relationship between concentrations of Lemon peels extract and DPPH radical scavenging activity. As the concentration increases, the inhibition percentage escalated significantly, showing that greatest concentration of Lemon peels extract being more active in counteracting free radicals. Present findings showed that ethanol and chloroform extracts of Lemon peels displayed antimicrobials effectiveness versus *S. aureus*, *P. aeruginosa*, and *C. albicans*, with different degrees of effectiveness. Using ethanol extract the IC<sub>50</sub> values for *S. aureus* was >2500 µg/ml, the IC<sub>50</sub> for *P. aeruginosa* was 838 µg/ml, and for *C. albicans* was 2194; and when using the chloroform ones the IC<sub>50</sub> for *S. aureus* was 602 µg/ml, the IC<sub>50</sub> of *P. aeruginosa* at 698 µg/ml and IC<sub>50</sub> for *C. albicans* at 482 µg/ml. The chloroform lemon peels extract seems to be more effectual overall compared with ethanol extract based on the IC<sub>50</sub> values. Conclusion: The present findings showed that *P. aeruginosa* is more susceptible to the ethanolic extract than *S. aureus* and *C. albicans*. While *C. albicans* exhibited the lowest IC<sub>50</sub> (482 µg/mL), revealing powerful antifungal activity for chloroform extract. Chloroform lemon peels extract seems to be more effectual overall compared with ethanol extract based on the IC<sub>50</sub> values.

**Keywords:** Lemon, Citrus limon, *S. aureus*, *P. aeruginosa*, *C. albicans*, lemon extracts.

## Introduction

Lemon (*Citrus limon*) be a member of Rutaceae family. It is considered one of the important plants with nutritional and medicinal importance, which is cultivating in wide range countries of world [1]. Different species of this plant were consumed in the Arabic conventional medicine as a sedative, analgesic, antiarrhythmic, a stomachic, along with skin caring [2, 3]. Peels of Citrus are rich with flavonoids glycosides, coumarin, sitosterol, as well as essential oils, which could be extracted and append to cosmetics and pharmaceutic products. Other bioactive compounds like terpenes, alcohols, aldehyde, and esters contribute to overall antimicrobial impacts of essential oils [4]. Moreover, citric acid and flavonoid, contributing to its antimicrobial activities, potentially presented naturally alternatives to synthetic antimicrobial agents [5].

Extracts of Lemon had long been known for their antimicrobial activities, making Lemon a subject of interest in the scientific research. Several investigations have revealed that lemon extracts exhibited inhibitory impacts on certain bacterial species such as *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Streptococcus pneumonia* (*S. pneumonia*), and *Escherichia coli* (*E. coli*), highlighting their potency in medical and food preservation applications [1, 6, 7]. Toan and Van revealed that lemon seed extracts effectively inhibited the growth of *Pseudomonas aeruginosa* (*P. aeruginosa*, multidrug-resistant bacteria accountable for hospital-acquired infections) at selectively applied concentrations [8]. Additionally, Jemikalajah et al. investigated the antibacterial impacts of lemon juice extracts against *P. aeruginosa* and *E. coli* and reported that lemon juice displayed concentration-dependent antibacterial impacts, with ethanolic extract showed stronger inhibition zones compared to aqueous extract [9]. The antimicrobial activity differs according to extract types and solvents, with methanol and ethanol extracts often exhibiting outstanding zones of inhibition. Lemon peel tend to be more effectual against Gram-positive bacteria, while antifungal activity against *Candida albicans* (*Candida albicans*) has also been emphasized, highlighting lemon's potential in developing alternative or complementary therapies for bacterial and fungal infections [1, 10].

*S. aureus*, *P. aeruginosa*, and *C. albicans* have been extensively studied due to their clinical importance, antibiotics resistance, and possible responsiveness to natural antimicrobial agents. *S. aureus* is Gram-positive bacteria commonly related with skin infections and antibiotic-resistant strains, and deems as a critical pathogen in healthcare settings [7]. *P. aeruginosa* is a Gram-negatives pathogen wellknown for its multidrug resistances pattern and ability to biofilms formation, engaged with critical hospital-acquired infections [9]. *C. albicans* is opportunistic fungal pathogen causes both superficial and systemic infections, especially in immunocompromised subjects [11]. Lemon peels contain bioactive compounds such as flavonoids, polyphenols, and essential oils, which have demonstrated antimicrobial properties against these pathogens [12]. This study aims to reveal its phytochemical profile, antioxidants efficiency and investigate the impacts of lemon extracts on a selected assortment of bacteria, including *S. aureus*, *P. aeruginosa*, and *C. albicans*, estimating their antimicrobial impacts and potential applications.

## Methodology

### Collection and Preliminary Processing of Lemon Peels for Extraction

Lemon fruits have been got from the local market and classified at College of Pharmacy, Branch of Pharmacognosy and Medicinal Plants. These fruits were thoroughly washed for removing dust, and then they peeled and spread out to dry at room temperatures up to fully dehydrated. Once being dried, peels have been finely ground by an electric grinder and stored in glass box until later use. Then lemon peel extract was prepared, and subsequent analysis had been conducted in the laboratories of Ghayat Al-Ma'rifa Company in Babylon province.

### Lemon Peels Extraction

Fifty gram of dried peel powder has been put in glass flask and dissolved in 500mL of 70% ethanol and left for 24 hrs. In further flask, equal weight of dried peel powder has been dissolved in 500mL of chloroform for the same period. Both flasks have been placed on the shaker for 6 hrs. to consolidate extraction. The yielded extracts were then filtered employing Millipore filter [13]. The resulted filtrates were posteriorly dried in an oven at 57-60°C, and obtained dried extract powder has been utilized for preparing ethanol and chloroform crude extracts.

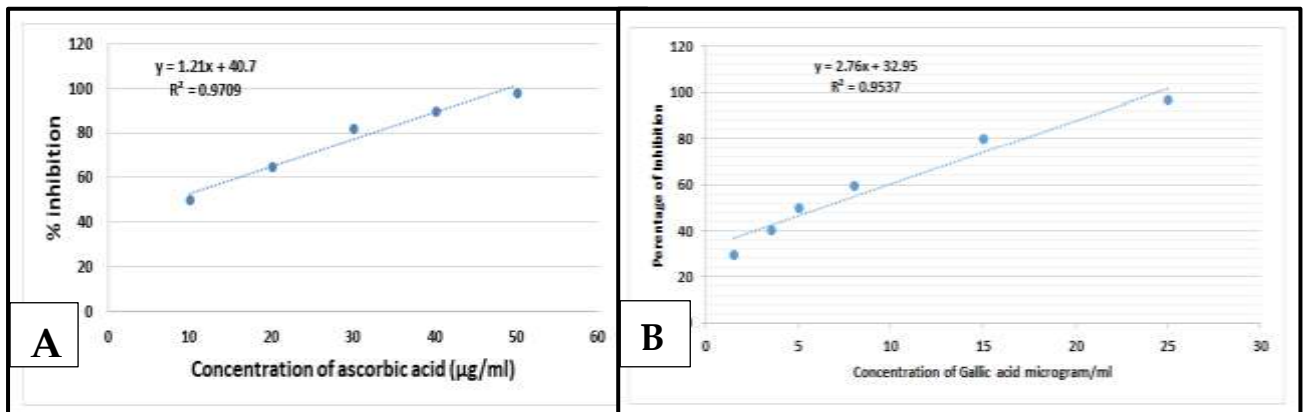
### Antioxidants Activities Estimation Applying DPPH Scavenging Assay

The electrons-donating capability of the samples and standards (gallic acid and vitamin-C) was assessed employing the bleaching influence of purple color ethanol solution of 2, 2--diphenyl--1--picrylhydrazyle (DPPH). The spectrophotometric technique has been applied employing stable DPPH radicals as a reagent. DPPH solution has been prepared at 0.002% concentration. Varied sample concentrations were added in distinct test tubes, and the ethanol has been utilized to adjust volume to 2mL. After that, 2mL of DPPH solution (ranged between 2.0- 0.001 mg/mL) has been appended to all test tubes. Subsequently, they incubated in darkness for 30 mins. The same technique has been used for vitamin-C and gallic acid. These samples were analysed in triplicate, and optical density (OD) was registered, at 517nm by the spectrophotometer. Ethanol with DPPH had utilized served as control [14]. Inhibition Percent of standards displayed in Figure (1); the formula that exercised for calculation is shown below:

$$\text{Inhibition of DPPH activity \%} = (A-B/A) * 100$$

Where: A = Optical density of control, B = Optical density of specimen.

**Figure (1):** Inhibition Percentage of standard (A: ascorbic acid, B: Garlic acid).



### Evaluation of flavonoids

The flavonoids were evaluated using spectrophotometric assay. Quercetin has been utilized to form the calibration curve [standard solutions 25, 50, 80, 100, 150, 200, 250 and 300 mg/100ml in 80% ethanol (v/v)]. The standard solutions or specimens (0.5 ml) were mixed with 1.5 ml 95% ethanol, 0.1ml 10% Al(NO<sub>3</sub>)<sub>3</sub>, 0.1ml of 1mol/l potassium acetate and 2.8ml D.W. In a blank, volume of Al(NO<sub>3</sub>)<sub>3</sub> has been replaced by same volume with D.W. After incubating at room temperatures for thirty mins, absorbance has been measured at 415nm. Flavonoids were expressed as Quercetin [15, 16].

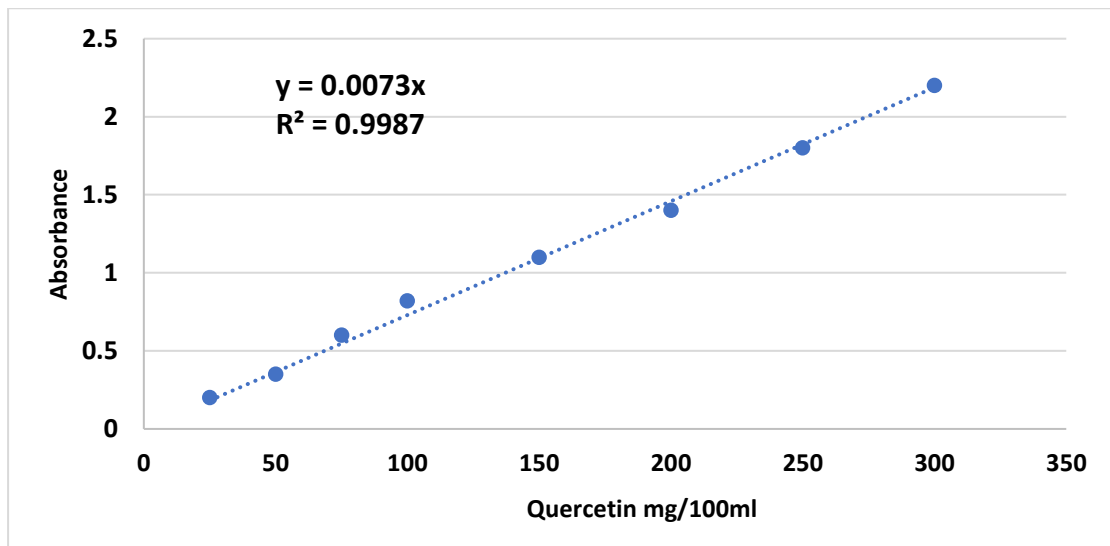


Figure (2): Standard curve of Quercetin mg/ml.

### Evaluation of Phenols

The total contents phenol of dried extracts was estimated employing Folin-Ciocalteu assay. A 1mL aliquot of sample (1mg/mL) was combined with 1mL of Folin-Ciocalteu's phenol reagent and allowed to react for 5mins. Subsequently, 10 mL of a 7% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution was appended, followed by 3mL of distilled deionized water (D.W), to ensure thorough mixing. This mixture has been incubated in darkness for 90 minutes at 23°C; after that, OD was measured at 761nm. Phenolic content has quantified by extrapolating data from the calibration curve that prepared utilizing gallic acid standard solution. This assessment has conducted in triplicate, and total phenolic content (TPC) has been expressed as milligram of gallic acid equivalent per gram of dried specimens [17, 18].

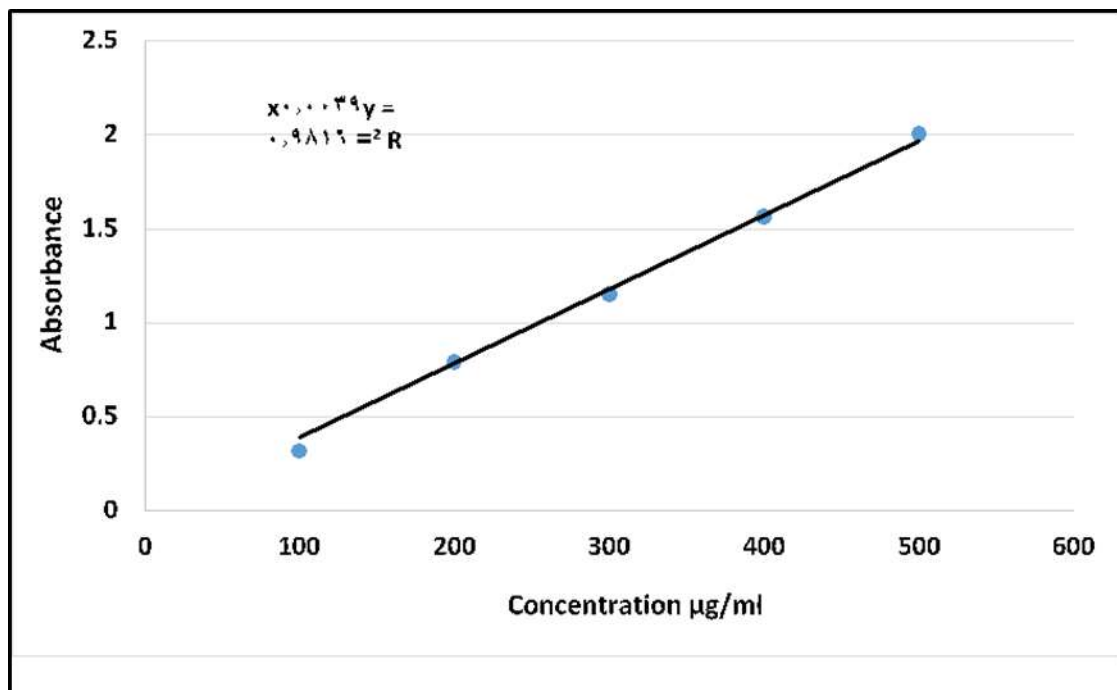


Figure (3): Standard curve of gallic acid mg/ml.

### Evaluation of Total Condensed Tannins Content

The tannin contents (or proanthocyanidin) have been assessed according to Broadhurst et al. (1978), with some modulation; catechin has been employed as a reference compound. About 400µL of

extracts has been appended to (3mL) vanillin solution (4%) (in methanol) and 1.5mL of concentrated HCl. Following incubated for 15 mins., OD value was recorded at 500nm. Condensed tannins were indicated as gE; Catechin.100g/DM [19, 20].

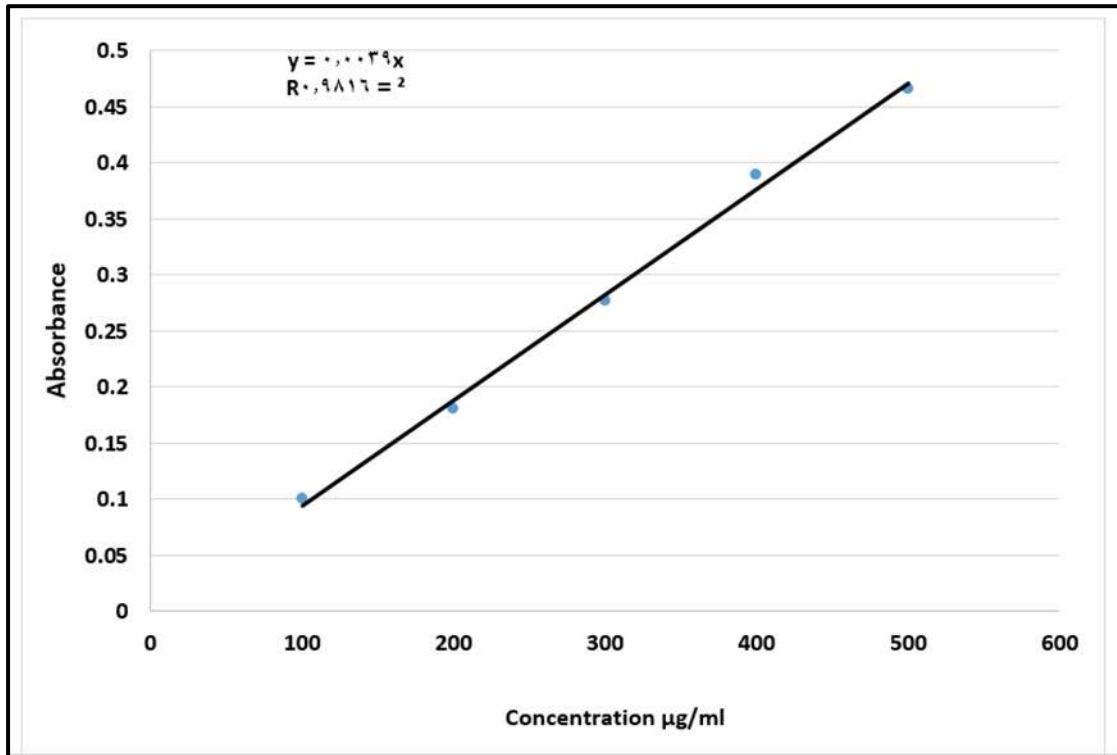


Figure (4): Standard curve for catechin mg/ml.

#### Evaluation of Total Alkaloids

##### Bromocresole Green Solution ( $1 \times 10^{-4}$ )

69.8 milligram of bromocresole green and 3 ml of 2-molar sodium hydroxide were mixed with 5ml of D.W up to fully dissolved, and then it was diluted with one liter with D.W.

##### Preparing of Phosphate Buffer (pH 4.7)

For preparing phosphate buffer with pH of 4.7, about 71.6 g of ( $\text{Na}_2\text{HPO}_4$ ) has been dissolved in 1L of D.W to produce a solution of 2 M. pH was adjusted to 4.7 by gradually add 0.2 M citric acid solution, which was prepared by mean of dissolve 42.02 gram of citric acid in 1L of D.W, up to required pH is achieved [21].

##### Standard Atropine Solution

1 mg of pure atropine was dissolve in 10ml of D.W. From standard atropine solution with volume of 0.2, 0.4, 0.6, 0.8, and 1ml were taken and carefully separated into sterilized glass tubes. 5ml of each phosphate buffer and BCG solution have added. Subsequently, 4ml of chloroform was gradually added with continual stirring. Then, 1 ml of Limon extract was placed into sterilized glazier tubes and added 5 ml of each phosphate buffer and BCG solution. After that, 4 ml of chloroform was added progressively, with continual blending. The fraction in chloroform layer is recorded at 470 nm [21].

##### High Performance Liquid Chromatography analysis

High performance liquid chromatography analysis (HPLC) system (Knauer company/Germany) has been employed to achieve the analysis. The Table (1) displays the components of this system. The separation and detection have been carried out according to study of Seal [22]. The mobile phase contains 1% aquatic. acetic acid solution (Solvent A) and acetonitrile (Solvent B), flow's rate was 1 ml/minute, temperature of column was adjusted at 28°C and injection volume was 20µl. The gradient elution has been achieved by changing the percent of solvent B to solvent A; and it has been

altered from 10-40%B in a linear pattern for period of 28 mins, from 40-60% B in 39 mins, from 60-90% B in 50 mins. The mobile phase is reconstituted to starting state (solvent B:solvent A:10:90) in 55 mins and let to work for a further 10 mins, prior injecting different specimen. The chromatogram of HPLC had been revealed utilizing the photo diode array UV detector at 210nm. Table (2) elucidates the gradient program.

**Table (1):** The components of HPLC system.

Components	Version	Companies and origins
Binary high pressure gradient pump	P6.1L	Knauer/Germany
Diod array detector	DAD 2.1L	
Sample loop (20µl) and injector	D1357	
Analysis & system controlling softwares	Claritychrom, V 7.4.2.107	Dataapex / Czech Republic

**Table (2):** The gradient program.

Time (mins)	Mobile A	Mobile B	Flow rate ml/mins
Zero	90%	10%	1
28	60%	40%	
39	40%	60%	
60	10%	90%	

The recognition of all compounds has been carried out by mean of matching times of retention and absorbance spectrum of each standard, concentration has been measured by sequent conc. of external standard substances to form calibration curve among conc. and its equivalent peak areas.

#### Preparation of bacterial suspension

The strains of *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 27853), and local isolate *C. albicans* were used to carried out the antimicrobial analysis of Lemon peels extracts.

The inoculation has been prepared from ten single colonies of 24 hours old MHA. These colonies have been transported to tubes contain 2mL sterilized water, followed by measuring absorbance at 600 nm employing the Biotek 800ST plate reader (Biotek/USA). Sterilized water has been added to the final 600nm of 0.236, which equal McFarland of 0.67. This suspension has been diluted 100-folds in MHB for obtaining final suspension of  $1 \times 10^6$  CFU/mL, 50µL of this suspension has been utilized for inoculating all wells, except the plank ones. The half-maximal inhibitory concentration (IC50) and minimum inhibitory concentration (MIC) were performed triplicate wells besides the positives and negatives control wells. Inhibition analysis has been carried out in the microplates of 96-well utilizing a final volume of 225µL, supplemented with plant extracts to achieve final conc. between 78-2500µg/ml. Microtiter plate (MTP) have been incubated for a night and measured for MIC as minimum concentration that revealed completed inhibitions of apparent growing [23].

#### The result

##### The phytochemical examination of lemon peels extract

Table (3) shows the phytochemical examination of lemon peels extract revealed that these peels have different bioactive compounds at different levels, the concentration of total polyphenol was 124 (mg/L), Flavonoids was at 150 (mg/L), Tannins level was 13 (mg/L), while alkaloids were found with low concentration at 5 (mg/L).

**Table (3):** The phytochemical content of lemon peels extract.

Name of active compound	Concentration (mg/L)
Total polyphenol	125

Flavonoid	151
Tannin	14
Alkaloid	6

The chemical analysis for bioactive compounds that found in lemon peels extract demonstrated the presence of polyphenol, flavonoids, tannin, and alkaloids, which participate in antimicrobial activity and antioxidant properties of Lemon peels. These compounds were excessively investigated for their prospects health impacts, as well as applications in medicine and food preservations. Polyphenols, present at a concentration of 124 mg/L, are well-known for their valuable antioxidants activity, which neutralize reactive oxygen species (ROS) and minimize oxidative stress [24]. Research had revealed that limon-derived polyphenols exhibited antimicrobial impacts against various bacterial and fungal pathogens, supporting their potential usage in natural antimicrobial agents [25]. Flavonoids, which detected at the level of 150 mg/L, are another major type of bioactive compounds of lemon peels. These compounds have anti-inflammatory, antibacterial as well as antifungal activities, making them beneficial in pharmaceutical applications (Fadhil et al., 2024) [26]. It is found that flavonoids from citrus fruits could inhibit bacterial growth and boost immune responses, thus consolidating their medicinal importance [24]. Tannins, found at 13 mg/L, also share in antimicrobial activity of lemon by damaging bacterial cell membranes and prohibiting microbial adhesion. Some studies support the antimicrobial role of tannins [27]; others suggested that their effectiveness based on concentration and extraction methods [28]. Alkaloids were found at levels of 5 mg/L in Limon peels, they were known for their pharmacological importance, including antimicrobial and anti-inflammatory activities. While some studies confirmed the existence of alkaloids in lemon peel extract, but did not detect the exact concentration [27, 29]; other study revealed that their relatively low concentrations in lemon peels might limit their direct antimicrobial activity [24].

#### DPPH radical scavenging Efficacy

Table (4) shows the association between concentrations of Lemon extract and DPPH radical scavenging activities.

**Table (4): DPPH % inhibition in lemon peels extract.**

Concentration $\mu\text{g/ml}$	5	10	25	50	60
DPPH % inhibition	17	33	46	61	80

#### DPPH radical scavenging Efficacy

The test of DPPH radical scavenging ability is widely utilized to estimate total antioxidant capacity of plant extracts. The findings indicated that lemon peels extracts exhibited significant antioxidant activity, where higher concentrations yielded higher inhibition proportion. At concentration of 60  $\mu\text{g/mL}$ , the extract showed 80% inhibition ability, emphasized its robust free radical neutralization potency. Yahya and Abid revealed that citrus peels exhibited dose-dependent antioxidant impacts, supporting the trend noticed in the current study [30]. Similarly, the present finding is in line with previous research of Rizaldy et al., which demonstrated that citrus peels contain high antioxidants activity due to its high content of polyphenolic and flavonoids [31]. However, some investigations suggested that antioxidants ability of lemon peels might differ depending on extraction procedure and solvents used. Gao et al. revealed that ethanol-extracts exhibited vigorous DPPH inhibition as compared with aqueous ones, indicating that the selection of solvent plays a definitive

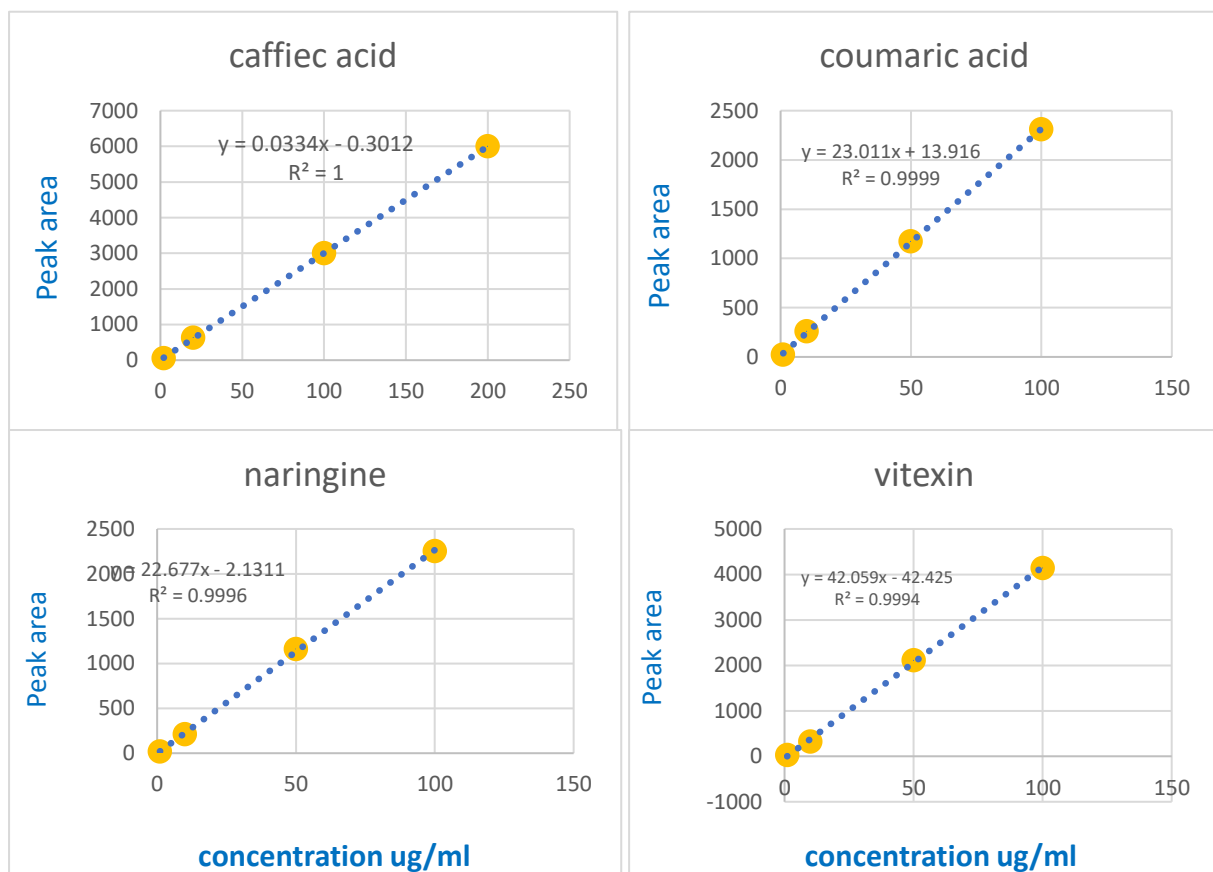
role in intensifying the antioxidants activity [32]. Furthermore, some studies argued that while citrus peels have antioxidants, their efficacy may not be as strong as other plants like extracts of green tea or grape seeds [25].

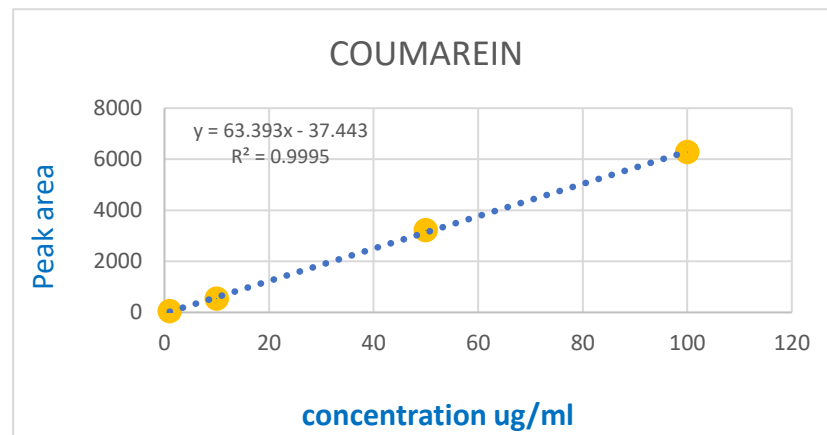
**Outcomes of High Performance Liquid Chromatography analysis**

The HPLC analysis of lemon extracts demonstrated that these extracts contain various bioactive compounds that including: caffeic acid; naringin, vitexin, coumarin and rutin, with varied concentrations and total yield as elucidated in Table (5). The standard curves of bioactive compounds shown in Figure (5).

**Table (5):** The outcomes of HPLC analysis.

Compounds	Peak area	Con. µg/ml	Total yield	µg/g material	plant
Caffiec acid	196.304	6.509	130.194	2.604	
Naringin	691.133	31.156	623.117	12.462	
Vitexin	282.826	9.073	181.464	3.629	
Coumarein	241.736	5.384	107.683	2.154	
Rutin	219.098	6.921	138.421	2.768	





**Figure (6):** Standard curves of bioactive compound.

The Caffeic acid, the known phenolic compound, exhibited a peak area of 196.304, a conc. 6.509  $\mu\text{g/ml}$ , and total yield of 130.194. The flavone glycoside, Vitexin is found at a peak area of 282.826, concentration of 9.073, with total yield of 181.464. These present measurements align with others gated by Aznar et al., who recorded similar concentrations of caffeic acid and Vitexin in citrus extracts and highlighted its antioxidant features and potential health advantages [33]. Regarding to Naringin, which is one of flavonoids commonly presented in citrus fruits, it is displayed a highest concentration among the assessed compounds, with a peak area of 691.133, the conc. at 31.156  $\mu\text{g/ml}$ , and a total yield of 623.117. This finding is coordinated with the study by Sowmya et al.; they identified naringin as one of predominant flavonoids in peels of citrus, especially in *Citrus maxima* [34]. Coumarin has various antimicrobial and anticoagulant impacts, was found at a peak area of 241.736, a conc. of 5.384  $\mu\text{g/ml}$ , and a total yield of 107.683. Our outcomes are supported by Aznar et al., who proven presence of coumarin in citrus [33]. The present study also agrees another studies, which confirmed the antimicrobial activity and anticoagulant properties of coumarin analogues and their roles in drug discovery [35, 36]. The present finding with regard to Rutin showed a peak area of 219.098, a conc. of 6.921  $\mu\text{g/ml}$ , and a total yield of 138.421; this compound is other flavonoids with vigorous antioxidants properties. Our finding is in line with previous studies that reported Rutin as a principle flavonoid in citrus peels and emphasized its role for protection against oxidative stress [34, 37]. Overall, these outcomes emphasize the existence of crucial bioactive compounds in the extracts of lemon, which support their potency applications in food, medicinal, and cosmetic manufactures.

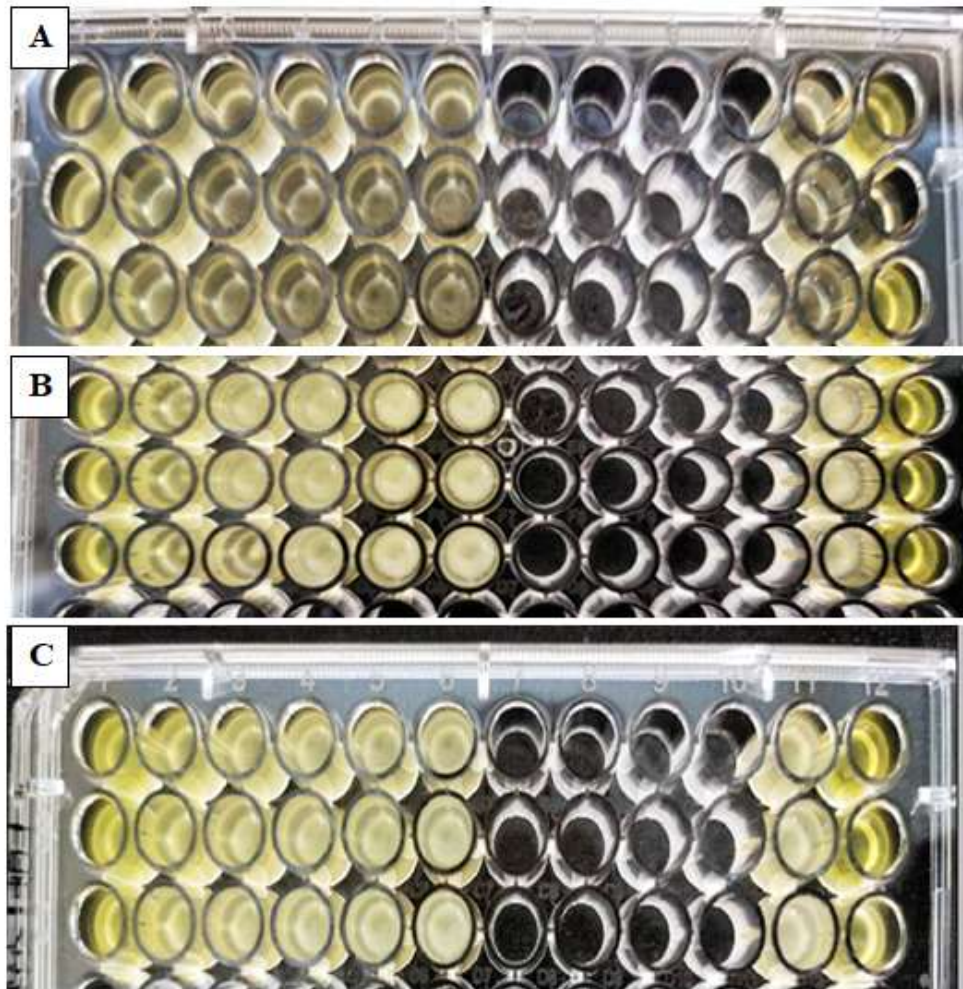
#### Antimicrobial analysis

##### Microbial Inhibition Employing of Lemon Peels Ethanol Extracts

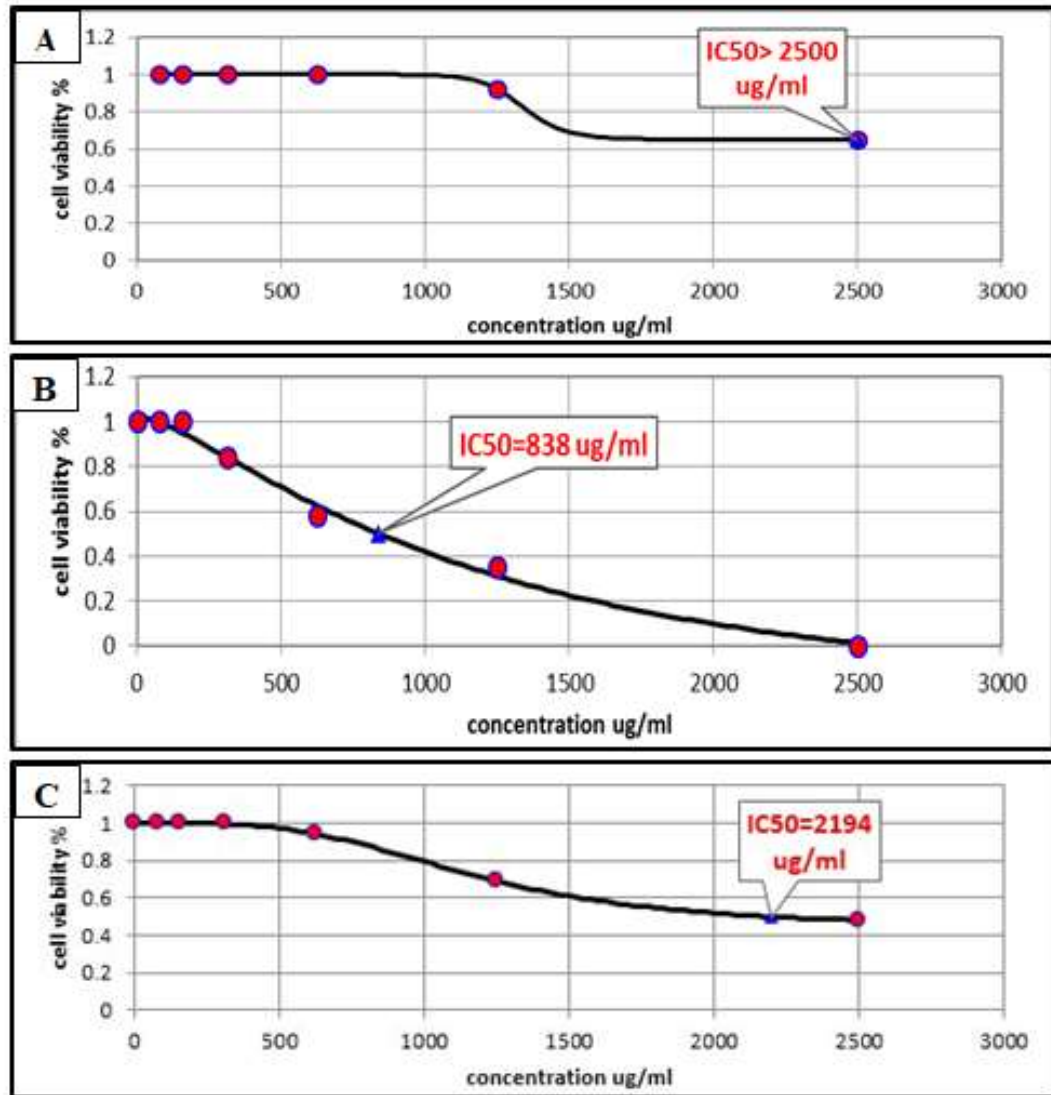
Table (6) reveals the Microbial Inhibition by Lemon peels ethanolic extract as expressed by MIC values; Figure (6) displays the detection of microbial inhibition using of Lemon peels ethanolic extracts. The IC<sub>50</sub> values of *S. aureus* is more than 2500  $\mu\text{g/ml}$ , IC<sub>50</sub> of *P. aeruginosa* is 838  $\mu\text{g/ml}$ , and to *C. albicans* was 2194  $\mu\text{g/ml}$  as shown in figure (7).

**Table (6):** Microbial Inhibition employing of lemon peels ethanol extracts.

Organism	MIC value $\mu\text{g/ml}$
<i>S. aureus</i>	>2500
<i>P. aeruginosa</i>	2500
<i>C. albicans</i>	>2500



**Figure (6):** Detection of Microbes Inhibition using of Lemon peels ethanolic extract: (A) *S. aureus*, (B) *P. aeruginosa*, and (C) *C. albicans*



**Figure (7):** The values of  $IC_{50}$   $\mu\text{g/ml}$  after using Lemon peels ethanolic extract for: (A) *S. aureus*, (B) *P. aeruginosa*, and (C) *C. albicans*.

Investigating Lemon extracts impacts as an antimicrobial agent on may provide valuable insights into alternative antimicrobial treatment protocol and natural therapeutic approaches. The inhibitory impact of lemon peel ethanol extracts on selected pathogens was estimated employing MIC and  $IC_{50}$  values.

The analysis of antimicrobial impacts of ethanol lemon peels extract against *S. aureus*, *P. aeruginosa*, and *C. albicans* demonstrated varied scores of activity. The MIC values of *S. aureus* and *C. albicans* was  $>2500 \mu\text{g/ml}$ , revealing that higher concentrations were required for successful inhibition, while MIC value for *P. aeruginosa* is  $2500 \mu\text{g/ml}$ , revealing a moderate susceptibility to Lemon ethanol extract. The current finding aligns with the study of Abdel-Salam and Mostafa, which reported that lemon peels extracts showed considerable antimicrobials effectiveness versus *S. aureus* & *P. aeruginosa*, with varied degrees of inhibition depends on concentration [38]. As well, Azali and Bakar reported that ethanol-based lemon peels extract revealed an antibacterial impact against *S. aureus*, which emphasized that citrus-derived compounds showed potent antimicrobial characteristics [39]. However, Dhanavade et al. reported different findings; they revealed that Lemon peels extracts showed lower inhibitory impacts against *P. aeruginosa*, indicating that antimicrobial activities may vary according to extraction method and mechanisms of bacterial resistance [5]. Also, current study conflicts with the study conducted by Yahya and Abid, which investigated the antimicrobial properties of Citrus peels extracts

and their silver nanoparticles versus *S. aureus*, *P. aeruginosa* as well as *C. albicans* and found that Gram-positive bacterial and fungal pathogens being more susceptible to citrus-derived silver nanoparticles than Gram-negative bacteria [30], whereas the results of our study indicated the opposite, as *P. aeruginosa* is more susceptible to ethanolic extract than *S. aureus*.

The values of IC50 were further demonstrated differences in microbial sensitivity. *S. aureus* exhibited an IC50 of more than 2500 µg/ml, suggesting a limited inhibition at tested concentrations and relative resistance to ethanol extract's impacts. *P. aeruginosa* showed an IC50 of 838 µg/ml, which indicates a potent response to ethanol extract and higher susceptibility as compared with *S. aureus*. The IC50 value for *C. albicans* was 2194 µg/ml, indicating moderate sensitivity to ethanol extract, which shows that it requires a relatively higher concentration for effective inhibition for effectual inhibition.

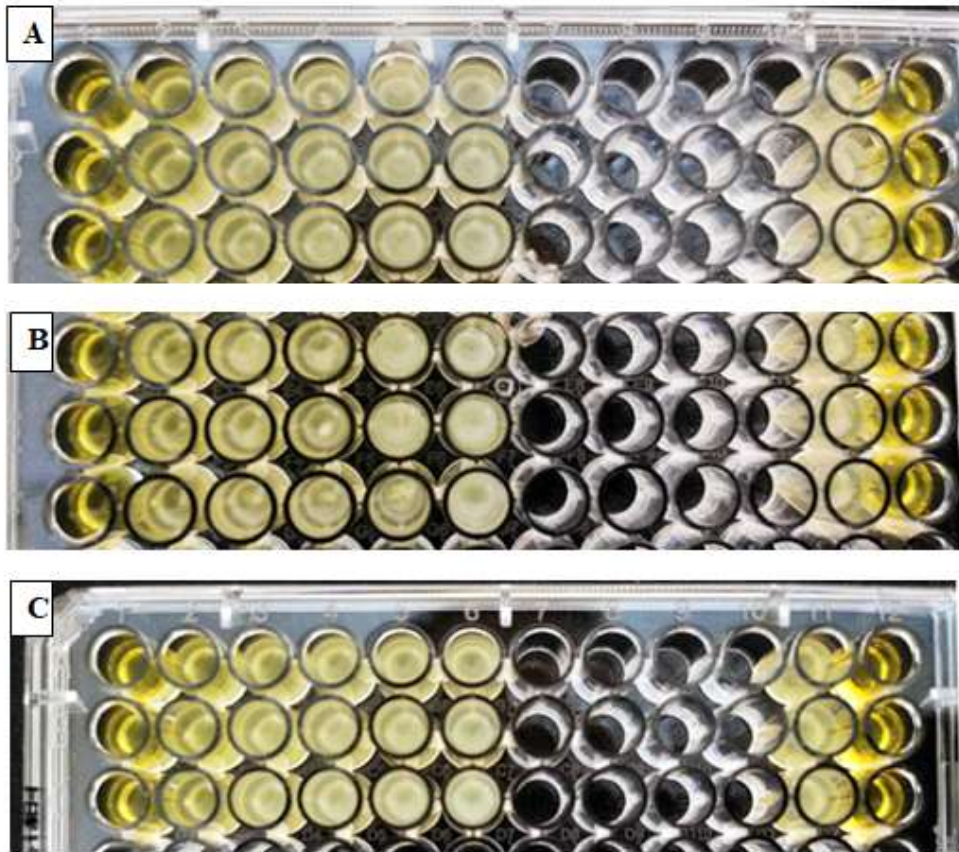
This finding reveals that ethanol lemon peels extract mayn't be highly effective against Gram-positive bacteria and *C. albicans* at lower concentrations. Our finding agrees with Nik Nur Shamiha et al., who demonstrated that citrus extracts have moderate antibacterial activity against *S. aureus*, but need higher concentrations for considerable inhibition [40]. However, another study has reported stronger antibacterial impacts of citrus extracts against *S. aureus*, when mixed with other antimicrobial agents or extracted with different solvents [41]. In contrast, IC50 for *P. aeruginosa* indicates a stronger antimicrobial impact. Our finding is in line with Dhanavade et al., who displayed that citrus peel extracts have remarkable antimicrobial activity against *P. aeruginosa*, possibly due to their content of flavonoids and essential oils that disrupting bacterial membranes [5].

#### Microbial Inhibition Employing Of Lemon Peels Chloroform Extracts

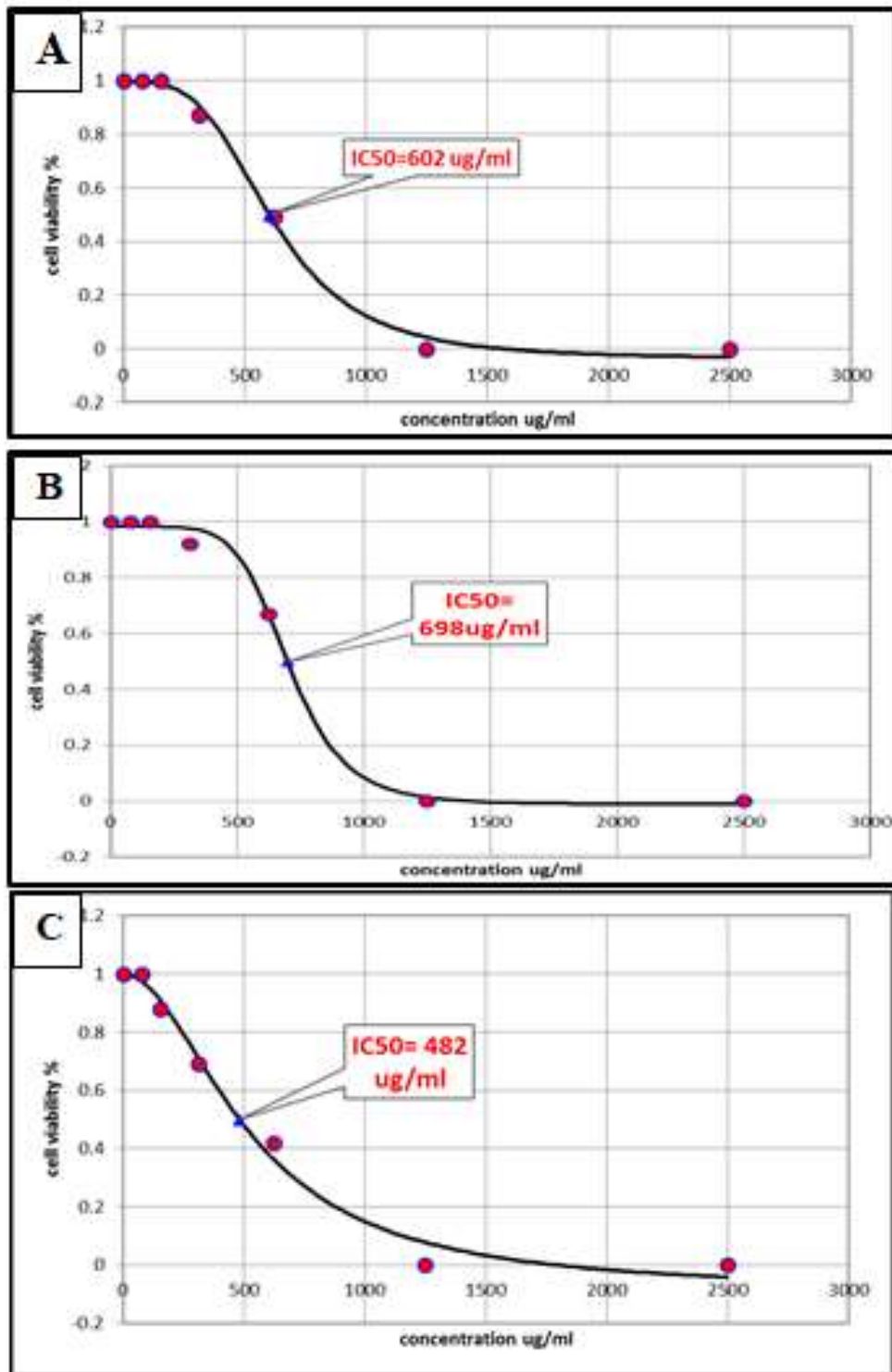
Table (7) reveals the Microbial Inhibition by Lemon peels Chloroform extract as expressed by MIC values; Figure (8) displays the detection of microbial inhibition using of Lemon peels Chloroform extracts. The IC50 values of *S. aureus* was 602 µg/ml, IC50 of *P. aeruginosa* was 698 µg/ml, and to *C. albicans* was 482 µg/ml as shown in figure (9).

**Table (7):** Inhibition of bacteria using of lemon peels chloroform extracts.

Organism	MIC value µg/ml
<i>S. aureus</i>	1250
<i>P. aeruginosa</i>	1250
<i>C. albicans</i>	1250



**Figure (8):** Detection of Microbes Inhibition using of Lemon peels chloroformextract: (A) *S. aureus*, (B) *P. aeruginosa*, and (C) *C. albicans*.



**Figure (9):** The values of IC50  $\mu\text{g/ml}$  after using Lemon peels ethanolic extract for: (A) *S. aureus*, (B) *P. aeruginosa*, and (C) *C. albicans*.

Using chloroform lemon peel extract as an antimicrobial agent versus *S. aureus*, *P. aeruginosa*, *C. albicans* showed uniform MIC value of 1250  $\mu\text{g/mL}$  in all studied microorganisms, which gives the impression that the bioactive compounds extracted utilizing chloroform reveal a consistent inhibitory impact, regardless the microbial classification. The chloroform lemon peels extract seems to be more effectual overall compared with ethanol extract based on the IC50 values, where the lower values indicated a stronger antimicrobial activity. *S. aureus* showed moderate susceptibility to chloroform extract; this is consistent with previous research that showed that lemon extracts have an antimicrobial

ability because they contain flavonoids and polyphenols [38]. The MIC value for *P. aeruginosa* proposing that while lemon peels extracts have antibacterial activities, Gram-negative bacteria may need a higher concentration or additional compounds for effectual inhibition [6]. Similarly, the MIC value for *C. albicans* was 1250µg/mL, indicating moderate antifungal impact, which returns to citrus bioactive compounds that could inhibit the synthesis of fungal cell wall [38]. The values of IC50 provide more support for our findings, with *C. albicans* exhibited the lowest IC50 (482 µg/mL), revealing powerful antifungal activity. The effective impacts of lemon against candida can be attributed to effects of terpenoids that have the ability of suppressing ergosterol's synthesis, which is one of fungal cell wall's constituents that maintain of permeability of cell membrane; additionally, essential oils of citrus lemon peel could impede the growth of *C. albicans* [10]. While some studies proposed that different concentrations and extraction techniques can yield more hopeful outcome, others investigations suggested that lemon extracts may not produce inhibition zones against *C. albicans* [11].

Similarly, *S. aureus* showed IC50 value of 602µg/mL, which proven the antibacterial potential of ethanol lemon peels extracts against Gram-positive bacteria. Abdel-Salam and Mostafa showed that citrus peel extracts revealed potent inhibition zones against *S. aureus*, supporting findings of our study. Lemon peels have several bioactive compounds like flavonoids, polyphenols, and essential oils, which have revealed antimicrobial capacities against these pathogens [38]. Conversely, *P. aeruginosa* showed highest IC50 (698µg/mL), indicates lower susceptibility to ethanol lemon peel extract. This is consistent with previous research suggesting that Gram-negative bacteria, especially *P. aeruginosa*, have intrinsic resistance mechanisms, including efflux pumps along with biofilms formation, which lessen plant-based antimicrobials effectiveness [9]. However, some studies have reported moderate antibacterial activity of citrus extracts against *P. aeruginosa* [42], suggested that higher concentrations or use another extraction methods may enhance efficacy.

### Conclusion

The present findings showed that ethanol and chloroform extracts of Lemon peels contain varied bioactive compound and have potent antioxidant properties. The current study demonstrated that solvent type significantly affects extraction competence and antimicrobial activity. Both extracts displayed antimicrobials effectiveness versus *S. aureus*, *P. aeruginosa*, and *C. albicans*, at different degrees of effectiveness. *P. aeruginosa* is more susceptible to the ethanolic extract than *S. aureus* and *C. albicans*. While *C. albicans* exhibited the lowest IC50 (482 µg/mL), revealing powerful antifungal activity for chloroform extract. Chloroform lemon peels extract seems to be more effectual overall compared with ethanol extract based on the IC50 values

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